J. King Saud Univ., Vol. **2**, *Agric. Sci.* (2), pp. 291-304 (1410/1990)

Scanning Electron Microscopy of Maize Pericarp and Associated Fungi after Treatment with Hot Mixture of Alcohol and Sodium Hypochlorite

Mohamed A. El-Meleigi

Plant Protection Department, College of Agriculture and Veterinary Medicine, King Saud University, Qassim branch, Buraydah, Saudi Arabia

Abstract. Dormant and germinated maize (*Zea mays* L.) kernels of Pioneer 3195 corn hybrid naturally infected with *Fusarium moniliforme, Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp. were surface sterilized and examined by scanning electron microscopy (SEM). The pericarp epidermal cells were found to be covered by a waxy layer that was ruptured upon germination of corn kernels. Numerous fungal conidia were observed lodged on or between the pericarp cells under a waxy layer adjacent to the silk remnant. Kernels dipped in a mixture (1:1) of 1% NaOCl and 95% ethanol at 75°C for 30 sec. were freed from the seedborne fungi. The waxy layer over the pericarp cells in the silk scar area was completely removed exposing collapsed fungal conidia after the previous treatment. The pericarp wax over the embryo and other parts of the kernel was either unaffected, cracked, or slightly scorched by heat treatment. Treatment with either hot ethanol or hot NaOCl solution did not eliminate the seedborne fungi and the pericarp waxy layer was moderately affected.

Introduction

A high percentage of maize (*Zea mays* L.) kernels are naturally contaminated or infected with several fungal species [1-4]. Kernels are externally contaminated with fungal propagules when infected stalks and leaves are crushed during mechanical harvest. Internal infection occurs during formation of corn kernels as a result of silk colonization by the pathogen and/or insect damage to the young tissues [2,3].

The number of seedborne fungi is commonly reduced but not eliminated by surface sterilization or hot water treatments [5-7]. The fungi isolated from maize kernels after surface sterilization are usually considered to represent pathogens that have colonized the internal tissues.

Treatment of infected maize kernels with 50% ethanol in 0.1% NaOCl at various temperatures and exposure times reduced but did not eliminate the seedborne

fungi [5,8]. However external and internal seedborne fungi, particularly *Fusarium moniliforme* Scheld., were eliminated when maize kernels when dipped in a mixture (1:1) of 1% NaOcl and 95% ethanol at 75°C for 30 sec. without affecting the viability of the seeds or seedlings [5,9].

The purpose of this study was to examine the effect of ethanol, sodium hypochlorite and heat treatments on the pericarp of corn kernel and associated fungi by scanning electron microscopy.

Materials and Methods

A seed lot of Pioneer 3195 corn hybrid, supplied by Pioneer Hi-Bred International Inc., Johnston, Iowa, U.S.A., with a high percentage (50%) of infection with *F. moniliforme* was selected for this study [8].

Treatments

Maize kernels were subjected to the following treatments under aseptic condition in a laminar flow hood:1- Not treated (control); 2- treated with 1% NaOCl for 2 min. then germinated under aseptic conditions for 72 hr; 3- soaked in a mixture (1:1) of 1% NaOCl and 95% ethanol at 75°C for 30 sec. (SET); 4- soaked in 0.5% NaOCl at 75°C for 30 sec., 5- soaked in 50% ethanol at 75°C for 30 sec.

Test for viable fungi

Seed samples from the various treatments were rinsed with sterile water, cut in half and Plated on acid potato dextrose agar pH 5 (APDA) and Komada media [10]. One hundred kernels from each treatment (fifty kernels/medium and 10 seeds/plate) were tested. Plates were incubated at room temperature for 7-10 days until seedborne fungi were ready for examination.

Preparation for SEM

In a laminar flow hood, corn kernels from the various treatments were cut in half and fixed in a mixture of formalin, acetic acid, and 50% ethanol (5:5:90) (FAA) for 72 hr. The kernel halves were then dehydrated in a graded series of ethanol in water: 50, 60, 70, 80, 95, and 100% for 1 hr each. The samples were critical point dried in a pressure bomb. The samples were mounted on stainless steel stubs and coated with gold on a sputter coater (Polar Instruments Inc., Doylestown, PA) with gold, then viewed on a JOEL Js M-35 scanning electron microscope.

Results

Viability of fungi

In spite of surface sterilization with 1% NaOCl for 2 min. the kernels of Pioneer 3195 were still infested with *F. moniliforme, Aspergillus* sp., *Trichoderma* sp. and

Penicillium sp. at rate of 50, 23, 9 and 8%, respectively. However corn kernels treated with a mixture of NaOC1 and ethanol at 75°C for 30 sec. were free from any seedborne fungi. Whereas, corn kernels treated with 0.5% NaOC1 or 50% ethanol at 75°C for 30 sec. were infected with *F. miniliforme, Aspergillus* sp. and *Penicillium* sp. at 23. 13 and 0.5% respectively.

SEM

SEM examination of the pericarp of untreated corn kernels showed the silk remnant attached to the base of the kernel and a large opening adjacent to the silk remnant (Plate1, Fig. 1). A roughened surface of the pericarp cells adjacent to the silk remnant was also observed (Plate 1, Fig. 1). The kernel pericarp cells were covered with smooth unbroken layer (Plate 1, Fig. 2). Upon germinating corn kernels for 24 hr, the smooth layer over the pericarp was ruptured and many fungal conidia appeared lodged between the folds of pericarp cells (Plate 1, Fig. 3). Fungal conidia were also observed at the opening (Plate 1, Fig. 4). Lemon shaped (3.2 × 4.8 μm) fungal conidia with rough surfaces were found in large numbers around the silk scar area on or under the waxy layer of the germinated seed (Plate 1, Figs. 5 & 6).

Chains of microconidia of *F. moniliforme*, developed from infected corn kernels were examined with SEM. The microconidia were 2-4 × 5-7 μ with rough surfaces (Plate 2, Fig. 7). Similar microconidia were observed lodged on the surface of the waxy layer over the embryo of dry kernels (Plate 2, Fig. 8). Cracks that were wide enough to accommodate fungal spores also were observed on the surface of dry kernels (Plate 2, Fig. 8). However, these cracks might have been formed during the preparation for SEM examination. Fungal spores similar to microconidia of *F. moniliforme* were also observed between the pericarp cells after the rupturing of the waxy layer at germination of corn kernels (Plate 2, Fig. 9). Other unidentified conidia were seen germinating between pericarp cells 72 hr after seed germination (Plate 2, Fig. 10). Fungal mycelia were also observed on the surface of pericarp cells of kernel germinated for 72 hr (Plate 2, Fig. 11). Chains of microconidia (1.7 × 2.6 μm) (*Penicillium* sp.?) were observed germinating on the pericarp cells near the scar of germinated kernels (72 hr) (Plate 2, Fig. 12).

Treating corn kernels with SET method caused the removal of the waxy layer from the pericarp in the silk scar area, thus fungal spores appeared collapsed on the naked cell walls of pericarp cells (Plate 3, Figs. 13 & 14). The waxy layer over the pericarp cells were partially removed by treating kernels with 50% ethanol at 75°C for 30 sec. (Plate 3, Figs. 15 & 16). Soaking kernels in 0.5% NaOCl at 75°C for 30 sec. had very little effect on the surface of the waxy layer on the pericarp (Plate 3, Figs. 17 & 18). Plate 4, Figs 19-24 show the effect of SET treatment on the waxy layer at various locations of the kernel surface. The wax was completely removed from the silk scar area exposing the rough surfaces of pericarp spherical cells (Plate 4, Fig. 19). At a location 2 mm from the micropyle, the waxy layer was partially removed and the

pericarp cells appeared elongated (Plate 4, Fig. 20). The wax over the central parts of the kernel endosperm and near the pedicel was only cracked or scorched (Plate 4, Figs. 21 & 22 & 23). The waxy layer over the embryo was apparently not affected by SET treatment and showed a distinct wavy pattern (Plate 4, Fig. 24).

The internal tissues of several corn kernels were examined with SEM. Physical evidence of internal fungal structures were not observed in this study.

Discussion

Isolation of fungi from surface sterilized corn kernels has been reported frequently [4,6,11]. The internal presence of seedborne fungi is easily recognized by plating surface sterilized corn seeds on nutrient media, but the location of infection has not been easily found. Attempts to observe internal fungal infection of corn kernel with SEM were rare and unsuccessful [12].

The present work revealed the presence of conidia belonging to 3 or 4 fungal species on surface sterilized corn kernels over and beneath the pericarp waxy layer. Two of these fungal spores resembled *F. Moniliforme* and *Penicilium* sp.. The surfaces of pedical pericarp cells were externally contaminated with fungal conidia. In similar study, *Aspergillus flavus* was observed to colonize and sporulate on corn silk surfaces [13].

A significant finding of our work was the observation of numerous fungal conidia under the waxy layer in the silk scar area. It is assumed that the silk was colonized with these fungi and conidia were deposited around the base of the silk over the forming corn pericarp.

These conidia were covered with soft wax at later stages of kernel maturation. Therefore, the fungal conidia remained protected from surface treatments. The germination of these conidia was also observed when corn seeds were germinated. The nature of the observed opening near the silk scar and its role in harboring fungal spores is not known.

The waxy layer in various parts over the pericarp of corn kernel appeared heterogeneous. This was concluded from examination of the wax surface after treatment with SET method. The wax over the embryo pericarp was barely affected by the treatments, whereas the micropyle area was completely removed, and associated fungal spores were collapsed. Using hot ethanol or NaOCl alone did not eliminate all seed borne fungi and the waxy layer was slightly affected. This suggests a role of the waxy layer in protecting seedborne fungi.

References

[1] Christensen, J.J. and Wilcoxson, R.D. "Stalk Rot of Corn." *Monograph No. 3, Amer. Phytopathol. Soc.* , St. Paul. Minn. (1966), 59

[2] Jones, R.K., Duncan, H.E., and Hamilton, P.B. "Planting Date, Harvest Date, and Irrigation Effects on Infection and Aflatoxin Production by *Aspergillus flavus* in Field Corn." *Phytopathology,* **71** (1981), 810-816.

[3] King, S.B. "Time for Infection of Maize Kernels by *Fusarium moniliforme* and *Cephalosporium acremonium,*" *Phytopathology,* **71** (1981), 796-799

[4] Marasas, W.F.O., Wehner, F.C., Van Kensburg, S.J. and Van Schalkwyk, D.J. "Mycroflora of corn Produced in Human Esophageal Cancer Areas." *Phytopathology,* **71** (1981), 792-796.

[5] El-Meleigi, M.A. "Method for Eradication of *Fusarium moniliforme.* from Maize Seeds." *Proc. Egyptian Soc. Appl. Microb.*, (1983), 179-186.

[6] Ikenbrry, R.W. "The Isolation of *Fusarium moniliforme* Sheld. form Corn Kernels." *Iowa Acad. Sci. Proc.*, **68** (1961), 100-102.

[7] Salama, A.M. and Mishricky, A.G. "Seed Transmission of Maize wilt Fungi with Special Reference to *Fusarium moniliforme* Sheld." *Phytopath. Z.*, **77** (1973), 356-362.

[8] El-Meleigi, M.A., Claflin, L.E. and Raney, R.J. "Effect of Seedborne *Fusarium moniliforme* and Irrigation Scheduling on Colonization of Root and Stalk Tissue, Stalk Rot Incidence and Grain Yields." *Crop Sci.*, **23** (1983), 1025-1028.

[9] El-Meleigi, M.A., Uyemoto, J. and Claflin, L.E. "A Method for Removing *Fusarium moniliforme* from Infested Corn Kernels." *Phytopathology,* **71** (Abstract). (1981), 215.

[10] Komada, H. "A New Selective Medium for Isolating *Fusarium* from Natural Soil." *Proceedings of American Phytopath. Soc.,* **3** (Abstract) (1976), 221

[11] Kucharek, T.A. and Kommedahl, T. "Kernel Infection and Corn Stalk Rot Caused by *Fusarium moniliforme. Phytopathology,* **56** (1966), 983-984

[12] Kaiser, R.P. "Initial Stages of Infection of *Zea mays* L. by *Fusarium moniliforme* Sheldon Emend." Snyd. & Hans. *Causing Stalk Rot. Ph. D. Thesis.* The Pennsylvania State University, University park. (1980), 100 p.

[13] March, S.F. and Payne, G.A. "Scanning EM Studies on Colonization of Dent Corn By *Asperigillus flavus.*" *Phytopathology,* **74** (1984), 557-561.

Plate 1. Scanning electronmicrograph of untreated Pioneer 3195 corn kernels showing:

Fig. 1. The surface of the pericarp, silk remnant (s) and a large opening (m) at the base (opposite side of the pedical) of a dormant kernel.

Fig. 2. The surface of pericarp cells in the silk scar area (× 1000).

Fig. 3. An opening (m) near the silk scar of germinated (24 hr) corn seed and disruption of the waxy layer (W) (× 100).

Fig. 4. Higher magnification of the pericarp of germinated corn kernels showing fungal spores (arrow) at the opening (m) and waxy materials (w) (× 1000).

Fig. 5. Pericarp cells near the micropyle and silk scar areas of germinated kernel showing fungal spores (arrows) lodged between pericarp epidermal cells (× 1000).

Fig. 6. Higher magnification of pericarp cells (c) of germinated kernels and an unidentified fungal spore (arrow) between two cells (× 5000).

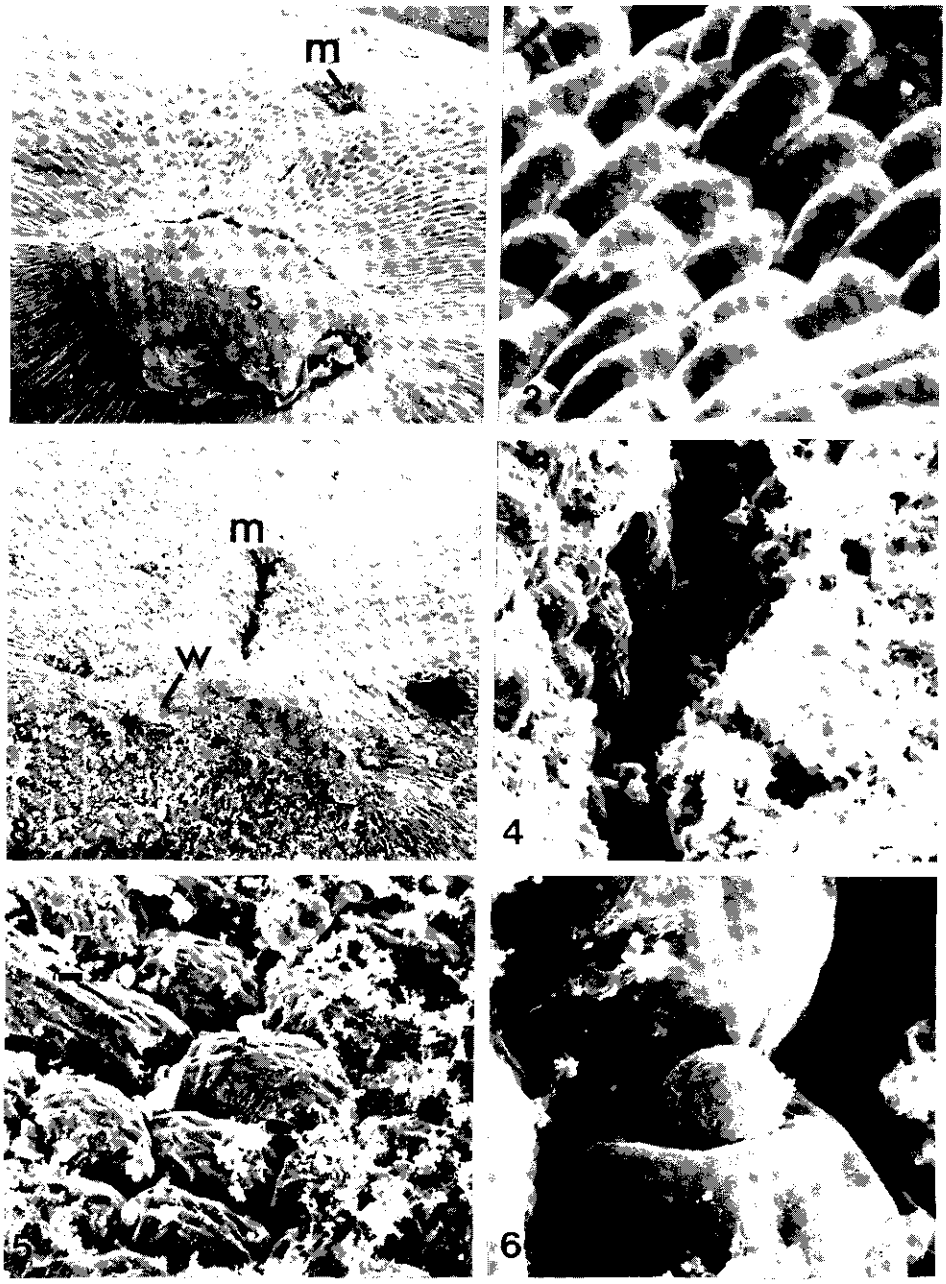

m
s
2
m
w
3
4
5
6

Plate 2. Fungal spores associated with Pioneer 3195 corn kernels:

Fig. 7. Microconidia of *Fusarium moniliforme* forming on corn kernels plated on media (× 5000).

Fig. 8. Microconidia of *F. moniliforme* (arrows) lodged over the embryo near a crack in the dry kernel (× 5000).

Fig. 9. *F. moniliforme* microconidium (arrow) between two pericarp cells near the silk scar area of germinated (24 hr) kernel (× 5000).

Fig. 10. Germinating fungal spore and formation of germ tubes (arrows) between pericarp cells of germinated (48 hr) kernels (× 5000).

Fig. 11. Fungal hypha (arrow) growing over the surface of germinated kernel (72 hr).

Fig. 12. Chains of fungal conidia and formation of germ tube (arrow) over the surface of pericarp cells adjacent to silk scar area of germinated kernel (48 hr).

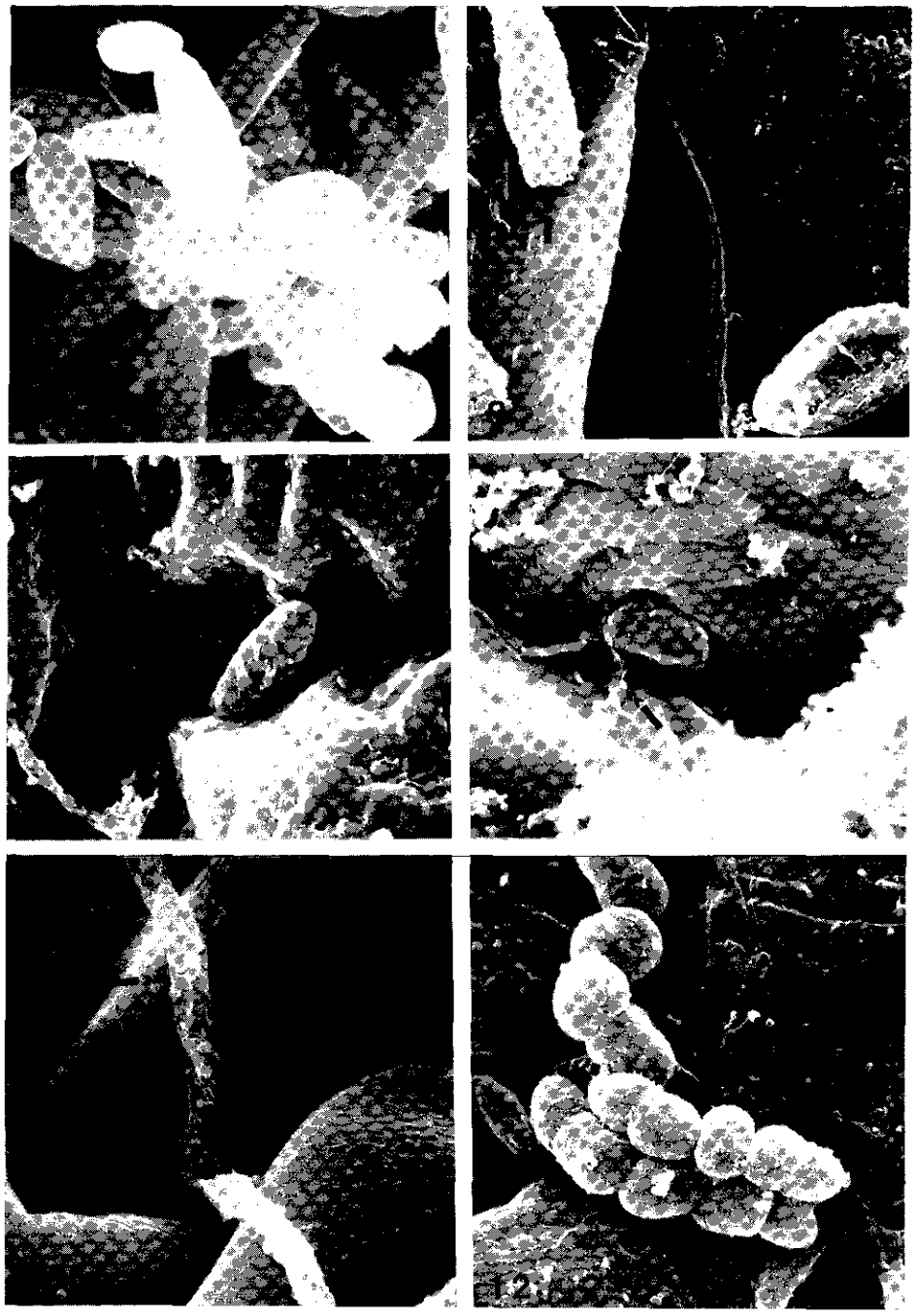


Plate 3. Effect of SET and other surface sterilization treatments on the surface of pioneer 3195 pericarp.

Fig. 13. Removal of the waxy layer from the pericarp cells in the silk scar area and collapse of fungal spores (arrows) after kernels were treated with a mixture (1:1) of 1% NaOCl and 95% ethanol at 75°C for 30 sec. (SET) (× 1000).

Fig. 14. Higher magnification from Fig 13 showing naked cell wall of pericarp epidermal cells (c) and collapsed fungal spores (arrows) (× 5000).

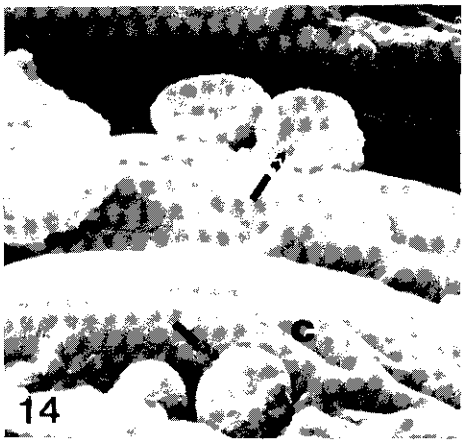
Fig. 15. The surface of pericarp after treatment with 50% ethanol, at 75×C, for 30 sec. showing partial removal of the waxy layer (W) and embedded fungal spores (arrows) (× 1000).

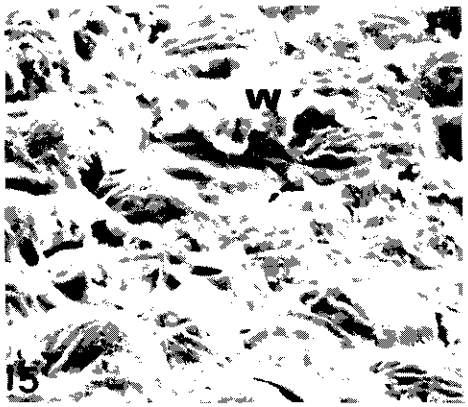
Fig. 16. Higher magnification of pericarp in Fig. 15 showing the partial removal of the waxy layer (W) and embedded fungal spores (arrow) and pericarp cells (C) (× 5000).

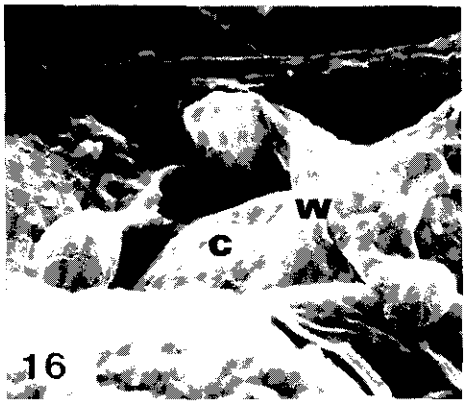
Fig. 17. Surface of pericarp cells treated with 0.5% NaOCl solution at 75°C for 30 sec. showing minor change in the surface of pericarp waxy layer (× 1000).

Fig. 18. Higher magnification of pericarp surface in Fig. 17 showing the surface of the waxy layer over the pericarp cells (× 5000).


13


c
14


w
15


w
c
16


17

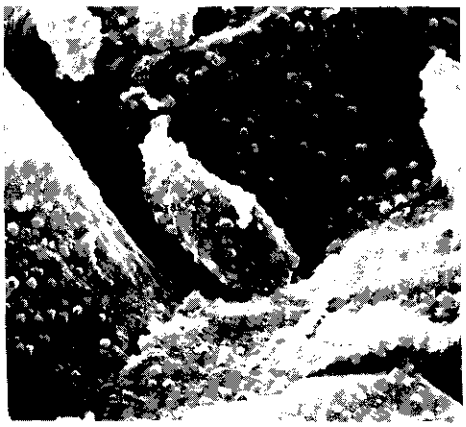


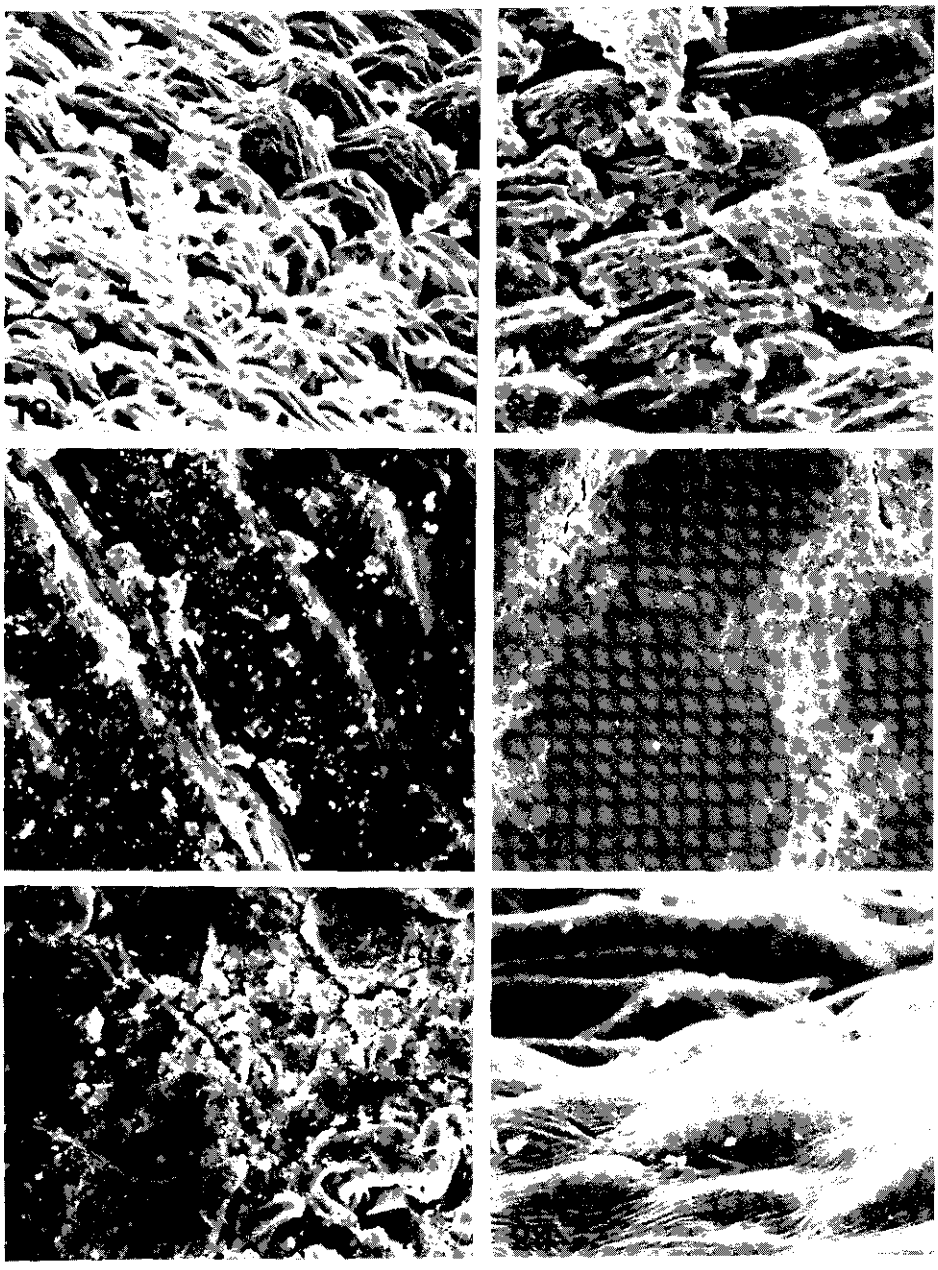
Plate 4. Effect of heating pioneer 3195 seeds with a mixture (1:1) of 1% NaOCl and 95% ethanol at 75°C for 30 sec. on the waxy layer over the pericarp.

Fig. 19. Surface of pericarp near the micropyle and silk scar area showing complete removal of the waxy layer and collapsed fungal spores (arrows) (° 1000).

Fig. 20. Pericarp cells over the endosperm at area located 2 mm from the micropyle showing the partial removal of the waxy layer (° 1000).

Fig. 21, 22 and 23. The waxy layer over the pericarp cells in center of opposite side of the embryo, side and near the pedical of corn kernel respectively, showing cracking but not removal of the wax (× 1000).

Fig. 24. The pericarp cells over the embryo with the waxy layer unaffected by heat treatment (× 1000).



تأثير الحرارة والكحول وهيبوكلوريت الصوديوم على سطح حبة الذرة والفطريات المصاحبة لها كما يظهر بواسطة المجهر الإلكتروني الماسح

محمد عبدالستار المليجي

قسم وقاية النبات، كلية الزراعة والطب البيطري، جامعة الملك سعود، فرع القصيم، بريدة، المملكة العربية السعودية

ملخص البحث. عقمت حبوب ذرة (*Zea mays* L.) جافة ونابتة من الهجين Pioneer 3195 مصابة بالفطريات *Aspergillus* sp. ، *Penicillium* spp, *Trichoderma* spp ، *Fusarium moniliforme* تعقيمًا سطحيًا ثم فحصت بواسطة المجهر الإلكتروني الماسح. وجدت الطبقة الخارجية للحبة مغطاة بطبقة شمعية والتي تمزقت مع عملية الإنبات حيث وجدت تحتها أعدادًا كبيرة من الجراثيم الفطرية مغمورة في الشمع أو بين الخلايا وذلك بالقرب من قاعدة الحبة وبقايا الحريرة المتصلة بها وأمكن القضاء على جميع الفطريات المصاحبة للحبة بغمرها في مخلوط ساخن (٧٥°م) من الكحول (٩٥٪) وهيبوكلوريت الصوديوم (١٪) (٨ : ١) لمدة ٣٠ ثانية وبفحص الحبوب المعاملة بالمجهر الإلكتروني شوهدت الجراثيم الفطرية محطمة والطبقة الشمعية قد أزيلت من منطقة القاعدة والحريرة دون المناطق الأخرى حيث لم تتأثر الشمعية في المناطق الأخرى أو تأثرت قليلاً نتيجة المعاملة بالمخلوط الساخن.